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# The Sweet Potato Hornworm, *Agrius convolvuli*, as a New Experimental Insect: Continuous Rearing Using Artificial Diets

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**ABSTRACT**—We developed a continuous rearing system for the sweet potato hornworm, *Agrius convolvuli*, for use as a new experimental insect for studies on insect biology. This species is closely related to the tobacco hornworm, *Manduca sexta,* which has occupied an important position as an experimental animal. By modifying the artificial diet for the silkworm, *Bombyx mori,* diets suitable for *Agrius convolvuli* were developed. The diets contained various amounts of sweet potato leaf powder as a substitute for mulberry leaf powder. Among the five different diets prepared, SPLP-25 and SPLP-20 (ca. 17–22% leaf powder) were suitable for the first four instar larvae, while SPLP-10 and SPLP-5 (ca. 4–9% leaf powder) for the final 5th instar. Although most of the 5th instar larvae fed and grew on a diet designated as SPLP-0 lacking leaf powder, larval life was prolonged and the resultant pupae were smaller. Year-round egg collection and rearing system were developed after a slight modification of the system devised for *Manduca sexta.* The sweet potato hornworm was successfully reared on the artificial diet for over 20 generations during the past 3 years under this system.

The insect is considered to be a good experimental animal suitable for comparative studies with other large lepidopteran insects, such as *Manduca sexta* and *Bombyx mori.*

## INTRODUCTION

Insects offer considerable advantages as experimental animals for studies on various biological phenomena. For example, studies involving more than 3,200 different insects were cited in the book series edited by G. A. Kerkut and L. I. Gilbert in 1985, which covered insect physiology, biochemistry and pharmacology[8]. Insects most frequently cited were the fruit fly, *Drosophila melanogaster*, the American cockroach, *Periplaneta americana*, the desert locust, *Locusta migratoria*, the tobacco hornworm, *Manduca sexta* and the silkworm, *Bombyx mori.*

*Bombyx* is obviously the most extensively studied lepidopteran insect. The wealth of information on *Bombyx* accumulated for the sericultural industry made it an ideal organism for research on genetics, embryology, physiology and biochemistry, etc., and innumerable contributions have been made with this insect by many researchers in different disciplines [14]. Yet it is important to conduct comparative studies on other lepidopterans as well.

The tobacco hornworm, *Manduca sexta,* is another well studied lepidopteran. It is a rather new experimental animal adopted in the 1970's after the development of a rearing system on artificial diet [5, 18]. Yet *Manduca* is now the most popular lepidopteran model, especially in the field of insect endocrinology and neurobiology (see reviews [10, 11, 15, 16]). Since *Bombyx* and *Manduca* are both large lepidopterans, each characterized by a different scientific background, it would be useful to carry out comparative studies between the two insects. However, as *Manduca* does not occur in Japan, our focus was directed to another related sphingid moth, *Agrius convolvuli.* The size and morphology are almost similar to those of *Manduca* (Fig. 1).

*Agrius convolvuli* is a cosmopolitan species widely distributed in the tropical,subtropical and temperate zones including Japan. Although it is one of the major defoliators of sweet potato crops, there are few descriptions on its life cycle and physiology [9, 12, 13, 19], and no information on its nutrition or suitable artificial diet. However, we observed that this species could be reared on a diet very similar to the artificial diet for the silkworm, *Bombyx mori.* Here we report the outline of the rearing system of the sweet potato hornworm, *Agrius convolvuli*, using artificial diets, which made it possible to rear the insect throughout the year for conducting research. The procedure has provided a continuous supply of the hornworm for the past 3 years.

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## MATERIALS AND METHODS

*Field collection of Agrius pupae*

The initial colony of the sweet potato hornworm, *Agrius convolvuli*, was reared in early September 1989. More than 300 pupae were first collected from the sweet potato field of the National Agriculture Research Center (Yawara farm) located at Tsukuba-gun, Ibaraki, Japan. The collected pupae were kept under a long day photoperiod (16L:8D) at 25±1°C and 70–80% RH. However, about 80% of the pupae died due to heavy infestation with parasitic flies (species undetermined). Most of the remainder developed without entering the diapause. The healthy pharate adults were transferred to a net cage (1.8×1.8×1.8 m) placed on the sweet potato field to collect eggs. A potted chrysanthemum and three plastic cups filled with a 25% sucrose solution were located at one corner of the cage to provide food sources. Hatched larvae from the obtained eggs were reared on the host plant leaves in a 20×28×6 cm plastic cage (14–20 larvae/cage during the first to fourth instar; 2–3 larvae/cage during the final fifth instar) under a long day photoperiod (16L:8D) at 25±1°C. The pupae obtained were used to develop an indoor egg collection method which was essential to devize a continuous rearing system. No wild individuals were introduced into the colony maintained thereafter.

*Indoor egg collection*

Like *Manduca, Agrius* moths are nocturnal, and mate and lay eggs only at night. It was observed that a sufficient number of eggs could be obtained even during the winter season by modifying the procedure for *Manduca* described by Yamamoto [17] and Bell and Joachim [4]. Pharate adults were placed in a tray on the floor of a wooden cage (73×85×115 cm) one day before adult emergence. The inside-surface of the cage was covered with black paper to prevent extra light reflection. The moths were kept under a photoperiod of 17L:7D at 26±1°C. During the dark period a low level of illumination (about 2–5 lux) was applied using a rheostat-controlled 6-watt incandescent bulb, since no fertilized eggs could be obtained under complete darkness. As food source, a 25% sucrose solution was placed in a plastic cup. A potted sweet potato plant was placed near the cup as an oviposition site. High humidity, over 70% RH, was usually maintained by using a wide water bath under the mesh floor. Sometimes during the winter season, additional moisture was supplied by a timer-controlled humidifier. Eggs were collected from the plant every morning, and incubated at 25±1°C and about 80% RH until hatching.

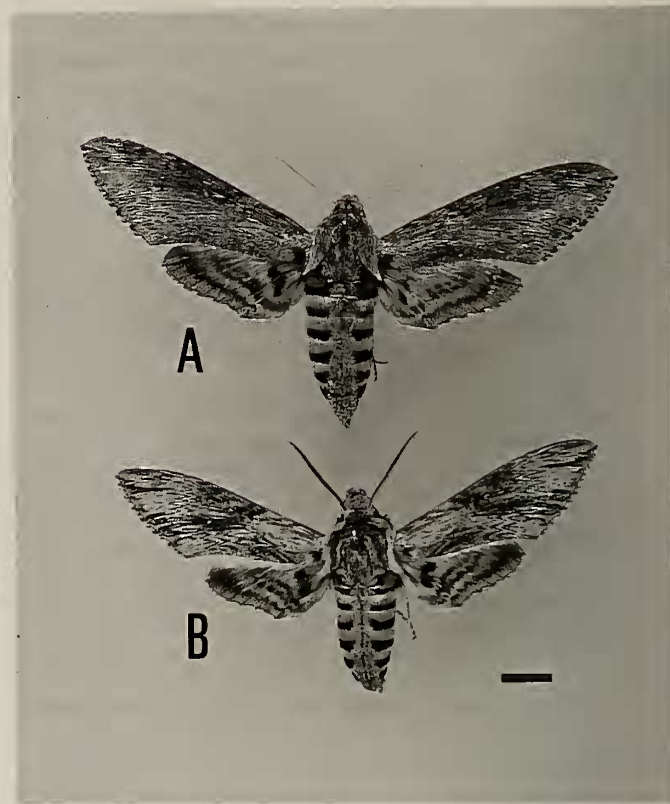


FIG. 1. Adult moths of the sweet potato hornworm, *Agrius convolvuli*. A,female; B,male. Bar=1 cm.

TABLE 1. Composition of artificial diets for the sweet potato hornworm, *Agrius convolvuli*

| Ingredient (dry matter) | Diet-A[1] (g) | SPLP-25 (g) | SPLP-20 (g) | SPLP-10 (g) | SPLP-5 (g) | SPLP-0 (g) |
|---|---|---|---|---|---|---|
| Mulberry leaf powder | 25.0 | — | — | — | — | — |
| Sweet potato leaf powder | — | 25.0 | 20.0 | 10.0 | 5.0 | — |
| Soybean meal | 36.0 [2] | 20.0 [3] | 25.0 [3] | 26.0 [3] | 28.0 [3] | 30.0 [3] |
| Agar | 7.5 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Potato starch | 7.5 | 10.0 | 10.0 | 12.0 | 13.5 | 15.0 |
| Sucrose | 8.0 | 10.0 | 10.0 | 12.0 | 13.5 | 15.0 |
| Cellulose powder | 20.8 | 29.6 | 29.6 | 34.6 | 34.6 | 34.6 |
| Soybean oil,not refined | 1.5 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| β-Sitosterol | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Sorbic acid | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Ascorbic acid | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Citric acid | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Wesson's salt mixture | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Total | 115.7 | 116.0 | 116.0 | 116.0 | 116.0 | 116.0 |
| Vitamin B mixture[4] | Added | Added | Added | Added | Added | Added |
| Antiseptics[5] | Added | Added | Added | Added | Added | Added |
| Distilled water(ml/g dry diet) | | | | | | |
| for 1–4th instar diet | 3.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| for 5th instar diet | 2.2 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |

1) Diet A: Standard diet for *Bombyx mori* developed by Horie *et al.* [7]; 2) Crude soybean meal, defatted; 3)Soybean meal, high nitrogen content (Solpea 600); 4)See Horie *et al.* [6]; 5)Antiseptics consisted of 0.015% (dry matter) of chloramphenicol and 0.75% (dry matter) of propionic acid.

*Preparation of artificial diets*

Artificial diets were developed after slight modifications of the diet for the silkworm, *Bombyx mori*, described by Horie *et al.* [7]. The diet composition is shown in Table 1. The main change was the substitution of sweet potato leaf powder for mulberry leaf powder. Five different diets designated as SPLP-0, SPLP-5, SPLP-10, SPLP-20 and SPLP-25 were prepared, each of which contained different amounts of sweet potato leaf powder. Mixed ingredients were stored in a refrigerator at 5°C. For the preparation of the diets, 300–400 ml of distilled water was added to 100 g of the mixed dry powder. The diet with a high water content (400 ml) was supplied to the first four larval instars, and that with a low content (300 ml) to the final 5th instar. After blending in the prescribed amount of water, the mixture was steamed for about 50 minutes at 100°C, then the diet was cooled down to room temperature. Each wet diet was covered with a wrapping film (Krewrap) and stored at 5°C until use.

*Rearing method on artificial diets*

A rearing system was developed after slight modifications of the system for *Manduca* adopted by L. M. Riddiford and J. W. Truman, University of Washington, Seattle. Namely, each newly hatched larva was individually confined in a plastic cup (50 ml) with a small piece of food (ca. 6 g). The diet was changed to a fresh one 7 days after feeding. When larvae molted into the 5th instar, each larva was transferred to a larger plastic cup (200 ml) with a lid on which several small circular holes were made to provide adequate aeration for the growing larva, and given a larger amount of food (ca. 25 g). Wandering larvae were transferred to another cup of the same size only with a piece of tissue paper, where they pupated. Throughout this experiment, eggs, larvae and pupae were kept in an environmental room maintained at 25±1°C, 50–60% RH, and under a long day photoperiod (16L : 8D).

## RESULTS

*Rearing on fresh host plant leaves*

Mean pupal weights of the field-collected *Agrius convolvuli* were 5.13±0.83 g for males (n=53) and 5.47±1.01 g for females (n=50). Hatched larvae from the obtained eggs were reared on fresh harvested leaves of sweet potato without difficulty (Table 2). Percentage of survival to the adult stage exceeded 90%. However this method required much space, large quantities of fresh sweet potato leaves, and time to handle the hornworms. The life cycle from egg to egg was

TABLE 2. Growth and development of *Agrius* larvae on the fresh sweet potato leaves

| | | |
|---|---|---|
| No. of eggs collected | | 255 |
| No. of hatched larvae | | 207 (82%) |
| No. of larvae placed on diets | | 207 |
| No. of larvae that molted into 5th instar | | 196 (95%) |
| No. of 5th instar larvae reared | | 163 |
| No. of individuals that became pupae | | 152 (93%) |
| No. of individuals that became adults | | 148 (91%) |
| Days from hatching to 4th larval ecdysis | | 12.5±0.8 (92)* |
| Days from 4th larval ecdysis to wandering | | 5.8±0.8 (65) |
| Days from wandering to pupation | | 4.2±0.5 (64) |
| Days from pupation to adult emergence | | 17.0±0.8 (60) |
| Pupal weight (g) | Male | 3.51±0.51 (89) |
| | Female | 4.01±0.72 (63) |

The rearing experiment was conducted in October 1989.
* Values are mean±S.D., and number of insects observed is indicated in parentheses.

TABLE 3. Growth and development of *Agrius* larvae on various artificial diets

| Diets used: 1–4th instar | Diets used: 5th instar | No. of larvae tested* | Pupation (%) | Duration of 5th instar (day) (Mean±S.D.) | Pupal weight (g) (Sex, Mean±S.D.) |
|---|---|---|---|---|---|
| *Early generation (July, 1990)* | | | | | |
| SPLP-25 | SPLP-25 | 36 | 97.2 | 5.7±0.8 | M 4.70±0.34 (20) ** |
| | | | | | F 5.26±0.45 (18) |
| SPLP-25 | SPLP-20 | 36 | 94.4 | 5.7±0.8 | M 4.72±0.41 (16) |
| | | | | | F 5.18±0.45 (15) |
| SPLP-25 | SPLP-10 | 36 | 100.0 | 5.8±0.7 | M 4.77±0.46 (19) |
| | | | | | F 5.23±0.49 (17) |
| SPLP-25 | SPLP- 5 | 35 | 91.4 | 5.8±0.6 | M 4.62±0.56 (10) |
| | | | | | F 5.18±0.62 (22) |
| SPLP-25 | SPLP- 0 | 36 | 91.7 | 6.9±1.2 | M 4.46±0.53 (18) |
| | | | | | F 4.79±0.93 (15) |
| *Advanced generation (July, 1993)* | | | | | |
| SPLP-25 | SPLP-25 | 59 | 100.0 | 5.2±0.4 | M 4.54±0.40 (34) |
| | | | | | F 5.11±0.30 (25) |
| SPLP-20 | SPLP- 5 | 52 | 100.0 | 5.0±0.5 | M 4.55±0.34 (23) |
| | | | | | F 4.96±0.55 (29) |

* Larvae were selected on day 0 of the 5th instar ( see text for detail).
** Number of insects observed is indicated in parentheses.

about 42 days at 25±1°C and 65–75% RH (roughly, egg: 4, larva: 18, pupa: 18, preoviposition: 2 days). Mean pupal weights were 3.51 g for males and 4.01 g for females, which were significantly smaller than those of the field-collected individuals.

*Rearing on artificial diets*

We prepared five different diets (Table 1) after slight modifications of an artificial diet for *Bombyx* larvae (Diet-A in Table 1). Preliminary experiments revealed that newly hatched larvae showed a high feeding activity on the SPLP-25 and SPLP-20 diets. Moreover, we could successfully rear the hornworm on the SPLP-25 diet throughout the larval stage and obtained two further generations of larvae. Also, we observed that the 5th instar larvae fed and grew well, even on the diets containing smaller amounts of host plant leaf powder. To determine the optimum regimen for the least expense, we carried out combination experiments of two different diets.

In July 1990, a total of 300 larvae were reared on the SPLP-25 diet until the molt to the 5th instar, then the 5th instar larvae were divided into several groups which were given different diets. As shown in Table 3, insects reared on the SPLP-25 diet throughout the larval stage showed a considerably higher performance in three developmental parameters examined than the individuals reared on fresh host plant leaves (Table 2). For example, the values for the mean pupal weights of the former were significantly larger than those of the latter in both sexes, although they were slightly smaller than those of the wild population. When 5th instar larvae were reared on the SPLP-0 diet, larval duration was prolonged by 1 to 2 days, and the resultant pupae were smaller. By contrast, the other groups reared on the SPLP-5, SPLP-10 and SPLP-20 diets did not show significant differences in the duration of larval life and in pupal weights when compared with those of the insects reared on the SPLP-25 diet.

Although the initial colony consisted of less than 50 pupae at the start, and no wild individuals were introduced thereafter, we have not yet observed any serious inbreeding depression. For example, the hatchability in the first generation which was 81% (Table 2) remained at the level of 70–90% after 20 generations. Also, the pupal weights were nearly the same in the early and the recent generations as shown in Table 3. Thus, viability has remained stable.

## DISCUSSION

We initially intended to identify an experimental insect suitable for comparative studies with the silkworm, *Bombyx mori*, which has been an important research target in our institute. Our attention was first directed to the tobacco hornworm, *Manduca sexta*, which has played an important role as an experimental insect. Since *Manduca* does not occur in Japan, we selected the sweet potato hornworm, *Agrius convolvuli*, a species closely related to *Manduca sexta*. As the morphology and life cycle of *Agrius* are similar to those of *Manduca*, the information accumulated on *Manduca* has been extremely useful in our attempt to develop an egg collection and rearing system. Actually, the system adopted for *Agrius* is basically the same as that for *Manduca*. Yet the composition of the artificial diet is very different from that developed for *Manduca*. We simply modified the diet for the commercial silkworm, *Bombyx mori*, by substituting sweet potato leaf powder for mulberry leaf powder. Our diets are satisfactory in nutritional requirements since we were able to rear the hornworm on the diet for over 20 generations during the past 3 years. During this time the hatchability of the eggs and survival rates did not change significantly, indicating that the diet is satisfactory for continuous rearing procedures.

However, there are a few problems which required further attention. First, it is preferable to develop a diet without host plant leaf powder. *Manduca* diets do not contain any leaf powder [1–3, 5, 18], while leaf powder is necessary for the current *Agrius* diet. At present we use SPLP-20 or SPLP-25 (ca. 17–22% leaf powder)for the 1st to 4th instar larvae, and SPLP-5 or SPLP-10 (ca. 4–9% leaf powder) for the last 5th instar. Yet it is noteworthy that most of the 5th instar *Agrius* larvae fed on the SPLP-0 diet lacking leaf powder survived, although their larval development was prolonged and the resultant pupae were smaller (Table 3). Therefore, we consider that an artificial diet without leaf powder could be developed through changes in the diet composition compatible with feeding activity. The second problem concerns the number of changes of diet necessary. In the *Manduca* rearing system, the diet is usually changed only once after a feeding larva reaches the 5th instar. By contrast, food must be changed twice in the *Agrius* rearing system to maintain an adequate larval development: first on the 7th day after hatching and second on the day when the larva molts to the 5th instar. To eliminate the first diet change, further improvement of the diets is required.

Needless to say, research on insects can be greatly facilitated by the development of a year-round rearing system. However, this system provides only one of the necessary conditions for a suitable experimental insect, and it is also important to accumulate fundamental information on the physiology and behavior of the insect. Unfortunately the sweet potato hornworm, *Agrius convolvuli*, had not been studied thoroughly until now. Yet the insect could be a suitable experimental insect as it is closely related to *Manduca sexta*. Presumably, the information accumulated on *Manduca* for the past 25 years will be useful for studies on the sweet potato hornworm. Studies on *Agrius* may also contribute to gain further insights into *Manduca* physiology and development.

Although both *Bombyx* and *Agrius* are large lepidopterans that are similar in many characteristics, they are also very different in various aspects. For example, the silkworm uses ingested nitrogen both for growth and synthesis of the silk proteins, and its diapause occurs at the embryonic stage. By contrast, the hornworm does not make silk, only builds a pupal chamber in soil, and enters diapause at the pupal stage. A comparative study of these developmental and behavioral differences would be most significant. We hope that such comparative studies among *Bombyx, Agrius* and *Manduca* will mutually contribute to a better understanding of the insect bio-mechanisms and functions.

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